Identification, Cloning, and Expression of Potential Diagnostic Markers for Q Fever

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ABSTRACT: The clinical diagnosis of Q fever is difficult. Whole cell antigens are currently used in several serological methods, but antigens are limited due to the hazardous nature of *Coxiella burnetii* cultivation. In this report, we described the method of detecting immunodominant antigens of *C. burnetii* by using proteomic techniques with patient sera, and cloning and expressing the selected antigens using a novel vector known for its ease of expression, purification, and downstream application.

KEYWORDS: Q fever; 2D gel electrophoresis; LC-MS-MS; protein antigens; biotin

INTRODUCTION

Coxiella burnetii, the etiologic agent of Q fever, is an obligate intracellular bacterium. *C. burnetii* is widely distributed in nature and infects a variety of mammals, birds, reptiles, fishes, and ticks.[1] Two phase variants of *C. burnetii* have been described, the highly virulent phase I and the less virulent phase II. In humans, infection is usually the result of inhalation of contaminated aerosols associated with infected sheep, goats, and, to a lesser extent, cattle. The extreme infectivity of the bacterium makes it a potential bioweapon and it is classified as a group B agent by the US Centers for Disease Control.

The diagnosis of Q fever is difficult and relies mainly on serological examination, the most commonly used method being indirect immunofluorescence assay (IFA).[2] Acute and chronic infections are characterized by different serological profiles.[3] Whole cell antigens are currently used in several serological methods, but are limited due to the hazardous nature of cultivation of *C. burnetii*. In this report, we identified six protein antigens by western blot analysis of whole cell lysate separated on a two-dimensional (2D) gel using patient sera that showed positive IgG by IFA against both phase I and phase II cells. The protein antigens were identified using a ProteomeX

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workstation (LC-MS) consisting of a liquid chromatography and a downstream electrospray ion trap mass spectrometer (LCQ DecaXP plus, Thermo Electrons). The identified antigens were cloned into a novel vector (pETAB′C). The clone gene was expressed as a tripartite protein fused with biotinylated leader peptide and ubiquitin. Affinity purification was carried out as described by Wang and colleagues.[4]

MATERIALS AND METHODS

Two-Dimensional Gel Electrophoresis and Western Blot Analysis

Phase I and Phase II *C. burnetii* (Henszering strain) cells were purified as described. The pure organisms were washed with cold PBS once and resuspended in lysis buffer (9 M urea, 4% CHAPS, and 50 mM dithiothreitol). Equal volume of sample solution (100 μg of protein) was subjected to 2D gel electrophoresis using Bio-Rad system as described by the manufacturer. The gel was transferred onto a polyvinylidene difluoride (PVDF) membrane and blotted against patient sera to identify protein antigens.

LC-MS Analysis to Identify Protein Antigens

The proteins that showed reactivity with patient sera were located in the gel and excised from the gel for LC-MS analysis. The digested proteins were analyzed as described.[5]

Cloning and Construction of Vectors Carrying Genes of Interest

PCR reactions were carried out using genomic DNA purified from phase II organisms (*C. burnetii* grown in VERO cells) and primer pairs of each gene containing *Not*I site on both the 5′ and 3′ overhang. The correct amplicons were cloned into the pETAB′C vector provided by Dr. Yang and plated on agar plate with ampicillin. Correct orientation of inserts was verified by PCR reaction and the sequence of inserted gene was confirmed.

Expression and Purification of Biotinylated Proteins

Escherichia coli BL21(DE3) cells transformed with respective plasmids were grown at 37°C overnight in instant TB medium (Novagen) supplemented with 50 μg/mL ampicillin and 2 M biotin. The purification was carried out as described by Wang and colleagues.[4]

RESULTS AND DISCUSSIONS

The combination of 2D electrophoresis, Western blot, and LC-MS allowed us to identify six antigens. Two of them (hsp 60 and Com-1) have been reported before and four (RecA, elongation factor Tu, OmpA-like transmembrane domain, and FtsZ) were newly identified. These antigens were successfully cloned into a pETAB′C vector and the expressed recombinant antigens were biotinylated in *E. coli* culture

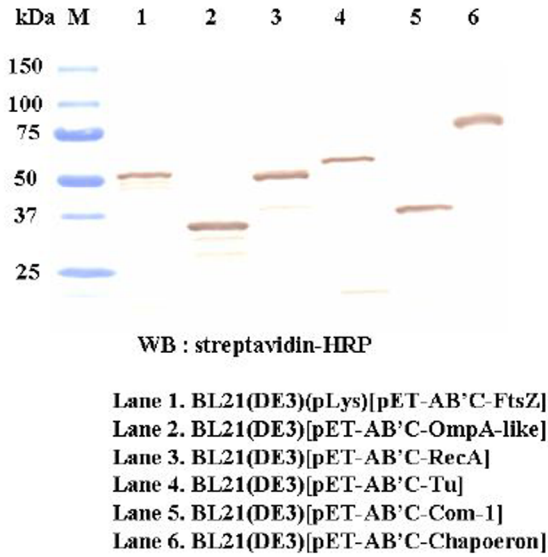

FIGURE 1. Western blot analysis of the expression of biotinylated protein antigens.

(FIG. 1). Highly pure expressed antigen was obtained using an avidin column with high efficiency. These biotinylated recombinant antigens can be used for the development of sensitive diagnostic assays.

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[*Conflict of interest statement:* The authors of this research declare that they have no conflict of interest.]

REFERENCES

1. SAWYER, L.A., D.B. FISHBEIN & J.E. MCDADE. 1987. Q fever: current concepts. Rev. Infect. Dis. **9:** 935–946.
2. TISSOT-DUPONT, H., X. THIRION & D. RAOULT. 1994. Q fever serology: cutoff determination for microimmunofluorescence. Clin. Diagn. Lab. Immunol. **1:** 189–196.
3. MAURIN, M. & D. RAOULT. 1999. Q fever. Clin. Microbiol. Rev. **12:** 518–553.
4. WANG, T., E. EVDOKIMOV, K. YIADOM, *et al*. 2003. Biotin-ubiquitin tagging of mammalian proteins in *Escherichia coli*. Protein Expr. Purif. **30:** 140–149.
5. CHAO, C.C., D. CHELIUS, T. ZHANG, *et al*. 2004. Proteome analysis of Madrid E strain of *Rickettsia prowazekii*. Proteomics **4:** 1280–1292.